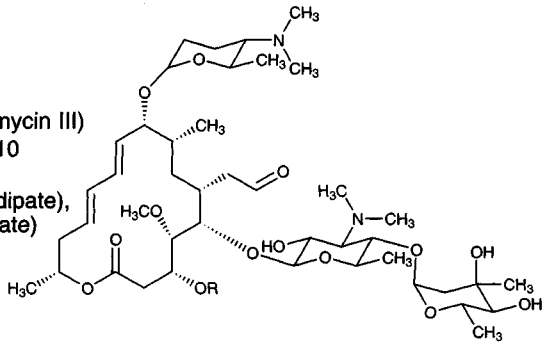
Spiramycin

Molecular formula: $C_{43}H_{74}N_2O_{14}$ (Spiramycin I), $C_{45}H_{76}N_2O_{15}$ (Spiramycin II), $C_{46}H_{78}N_2O_{15}$ (Spiramycin III)
Molecular weight: 843.07 (Spiramycin I), 885.10 (Spiramycin II), 899.13 (Spiramycin III)
CAS Registry No.: 8025-81-8, 24916-50-5 (I adipate), 24916-51-6 (II diacetate), 24916-52-7 (III diacetate)
Merck Index: 8904



Spiramycin I R = H
Spiramycin II R = $COCH_3$
Spiramycin III R = $COCH_2CH_3$

SAMPLE

Matrix: tissue
Sample preparation: Condition a 1 mL 100 mg Bond-Elut diol SPE cartridge with 1 mL chloroform (Caution! Chloroform is a carcinogen!). Mix 2 g minced muscle tissue with 800 μL water. Stir, vortex for 1 min at maximum speed, let stand for 15 min. Add 2 mL pH 8 buffer, mix briefly, add 10 mL chloroform. Stir at 100 rpm for 15 min, centrifuge at 4000 g for 10 min, discard the aqueous layer, filter the chloroform layer through glass wool. Add the filtrate to the SPE cartridge, wash with 500 μL chloroform, dry under vacuum, elute with three 200 μL portions of MeOH:100 mM ammonium acetate 50:50, inject a 200 μL aliquot of the eluate. (Buffer was 33.46 g K_2HPO_4 and 1.046 g KH_2PO_4 in 1 L water.)

HPLC VARIABLES

Guard column: 4 × 4 5 μm C18
Column: 125 × 4 5 μm Lichrospher RP18
Mobile phase: Gradient. A was MeCN. B was MeOH. C was 0.1% trifluoroacetic acid in water. A:B:C from 20:20:60 to 25:55:20 in 10 (?) min
Flow rate: 0.5
Injection volume: 200
Detector: MS, HP Model 5989 A, desolvation chamber 60°, source 280° and 300° in negative and positive chemical ionization mode, respectively, with methane as reagent, quadrupole 100°, particle beam nebulizer helium 345 kPa, scan m/z 475.3-684.4 in NCI and 843.5-684.4 in PCI

CHROMATOGRAM

Retention time: 5.2
Limit of detection: 50 μg/kg

OTHER SUBSTANCES

Extracted: erythromycin, josamycin, tilmicosin, tylosin

KEY WORDS

muscle; cow; SPE

REFERENCE

Delépine,B.; Hurtaud-Pessel,D.; Sanders,P. Multiresidue method for confirmation of macrolide antibiotics in bovine muscle by liquid chromatography/mass spectrometry, *J.AOAC Int.*, **1996**, *79*, 397–404.

SAMPLE

Matrix: tissue
Sample preparation: Condition a 500 mg Bond Elut SCX SPE cartridge (Varian) with 5 mL MeOH and 10 mL 100 mM pH 4.4 KH_2PO_4 buffer. Homogenize 5 g tissue with 100 mL MeOH: 0.3% metaphosphoric acid 30:70 at high speed for 2 min, filter through 2 mm Hyflo Super-Cel coated on a suction funnel (when filtering liver or kidney add several grams of Hyflo Super-Cel to the homogenized solution before filtration). Evaporate the filtrate to ca. 20 mL under

reduced pressure at 45°, add to the SPE cartridge, wash with 10 mL distilled water and 5 mL 100 mM pH 8.9 K_2HPO_4 buffer, elute with 10 mL MeOH, evaporate the eluate to dryness under reduced pressure at 45°, dissolve the residue in 1 mL MeCN:50 mM pH 4.5 NaH_2PO_4 buffer 30:70, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 150 × 4.6 5 μm Puresil 5C18 (Waters)
Mobile phase: Gradient. A:B from 60:40 to 0:100 over 16 min. A was buffer. B was MeCN:buffer 40:60 (Buffer was 2.5 g KH_2PO_4 dihydrate and 0.65 mL 85% phosphoric acid dissolved in 1 L distilled water, pH 2.5.)
Column temperature: 35
Flow rate: 1
Injection volume: 10
Detector: UV 232 for 9 min, UV 287 for 2 min, UV 232 for 4 min

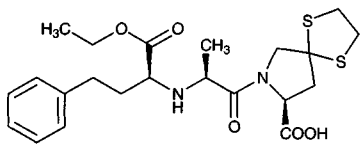
CHROMATOGRAM
Retention time: 3.23
Limit of detection: 50 ng/g

OTHER SUBSTANCES
Extracted: josamycin, leucomycin (kitasamycin), mirosamicin, tylosin

KEY WORDS
meat; SPE

REFERENCE
Horie,M.; Saito,K.; Ishii,R.; Yoshida,T.; Haramaki,Y.; Nakazawa,H. Simultaneous determination of five macrolide antibiotics in meat by high-performance liquid chromatography, *J.Chromatogr.A*, **1998**, *812*, 295–302.

Spirapril



Molecular formula: $C_{22}H_{30}N_2O_5S_2$
Molecular weight: 466.62
CAS Registry No.: 83647-97-6, 94841-17-5
Merck Index: 8905
Lednicer No.: 4 83

SAMPLE
Matrix: solutions

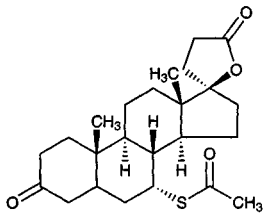
HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee RP-18
Mobile phase: MeCN:water 50:50 containing 0.5 mM tetramethylammonium hydroxide, adjusted to pH 2
Column temperature: 70
Detector: UV 217

CHROMATOGRAM
Retention time: 3.6

KEY WORDS
comparison with capillary electrophoresis

REFERENCE
Steuer,W.; Grant,I.; Erni,F. Comparison of high-performance liquid chromatography, supercritical fluid chromatography and capillary zone electrophoresis in drug analysis, *J.Chromatogr.*, **1990**, *507*, 125–140.

Spironolactone



Molecular formula: $C_{24}H_{32}O_4S$
Molecular weight: 416.58
CAS Registry No.: 52-01-7
Merck Index: 8917
Lednicer No.: 1 206

SAMPLE
Matrix: blood
Sample preparation: Add 100 μL 500 ng/mL methyltestosterone in MeCN to 100 μL serum, vortex for 15 s, centrifuge at 5000 rpm for 3 min, inject 100 μL of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 5 μm Spherisorb ODS-2
Column: 250 × 4 5 μm Spherisorb ODS-2
Mobile phase: MeOH:water 67:33
Flow rate: 1
Injection volume: 100
Detector: UV 238

CHROMATOGRAM
Retention time: 6.61
Internal standard: methyltestosterone (12.65)

OTHER SUBSTANCES
Extracted: metabolites

KEY WORDS
rat; serum

REFERENCE
Kaukonen,A.M.; Vuorela,P.; Vuorela,H.; Mannermaa,J.-P. High-performance liquid chromatography methods for the separation and quantitation of spironolactone and its degradation products in aqueous formulations and of its metabolites in rat serum, *J.Chromatogr.A*, **1998**, *797*, 271–281.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Bond-Elut C18 SPE cartridge with two 2.5 mL portions of MeOH and two 2.5 mL portions of water. Condition a Bond-Elut CN SPE cartridge with two 2.5 mL portions of MeOH and two 2.5 mL portions of hexane. Plasma. 1 mL Plasma + 100 μL 1 μg/mL IS in water + 1 mL water, add to the C18 SPE cartridge, wash with two 1.5 mL portions of water, wash with 1 mL MeOH:water 40:60, elute with two 1 mL portions of MeCN. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 100 μL ethyl acetate, add 1.5 mL hexane, mix, add to the CN SPE cartridge, wash with 2 mL hexane, elute with two 750 μL portions of MeCN. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot. Urine. 1 mL Urine + 1 mL IS in water, add to the C18 SPE cartridge, wash with two 1.5 mL portions of water, wash with 1 mL MeCN:water 35:65, elute with two 1 mL portions of MeCN. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 100 μL ethyl acetate, add 1.5 mL hexane, mix, add to the CN SPE cartridge, wash with 2 mL hexane, elute with two 750 μL portions of MeCN. Evaporate the eluate to dryness under reduced pressure, reconstitute the residue in 200 μL mobile phase, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 125 × 4.9 5 μm Spherisorb S5 ODS1
Mobile phase: MeOH:water 65:35, pH adjusted to 3.4 with phosphoric acid (plasma) or Gradient. MeOH:water acidified to pH 3.4 with phosphoric acid from 55:45 to 65:35 over 10 min (concave curve). (urine)

Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Retention time: 3.0 (plasma), 8.3 (urine)
Internal standard: 16α,17α-epoxyprogesterone (7 (plasma), 14 (urine))
Limit of quantitation: 31.25 ng/mL (urine), 6.25 ng/mL (plasma)

OTHER SUBSTANCES
Extracted: metabolites, canrenone

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Varin,F.; Tu,T.M.; Benoît,F.; Villeneuve,J.P.; Théorêt,Y. High-performance liquid chromatographic determination of spironolactone and its metabolites in human biological fluids after solid-phase extraction, *J.Chromatogr.*, **1992**, *574*, 57–64.

SAMPLE
Matrix: blood, urine
Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 μL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) μL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES
Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 μm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 239.3

CHROMATOGRAM
Retention time: 20.68

KEY WORDS
whole blood

REFERENCE
Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE
Matrix: solutions
Sample preparation: Prepare spironolactone solutions in MeCN:water 50:50 containing 2 μg/mL testosterone, inject a 30 μL aliquot.

HPLC VARIABLES
Guard column: 20 × 3.9 4 μm Sentry guard column (Waters)

Column: 150 × 3.9 4 μm mean pore diameter 60 Å Nova-Pak Phenyl
Mobile phase: THF:water 21.5:78.5
Flow rate: 1
Injection volume: 30
Detector: UV 250

CHROMATOGRAM
Retention time: 39.5
Internal standard: testosterone (22.5)

OTHER SUBSTANCES
Simultaneous: degradation products

REFERENCE
Kaukonen,A.M.; Vuorela,P.; Vuorela,H.; Mannermaa,J.-P. High-performance liquid chromatography methods for the separation and quantitation of spironolactone and its degradation products in aqueous formulations and of its metabolites in rat serum, *J.Chromatogr.A*, **1998**, *797*, 271–281.

SAMPLE
Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 60:1.5:0.5:38
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM
Retention time: k' 3.42

REFERENCE
Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 250 × 4.6 5 μm Supelcosil LC-DP (A) or 250 × 4 5 μm LiChrospher 100 RP-8 (B)
Mobile phase: MeCN:0.025% phosphoric acid:buffer 25:10:5 (A) or 60:25:15 (B) (Buffer was 9 mL concentrated phosphoric acid and 10 mL triethylamine in 900 mL water, adjust pH to 3.4 with dilute phosphoric acid, make up to 1 L.)
Flow rate: 0.6
Injection volume: 25
Detector: UV 229

CHROMATOGRAM
Retention time: 8.50 (A), 9.12 (B)

OTHER SUBSTANCES
Also analyzed: acebutolol, acepromazine, acetaminophen, acetazolamide, acetophenazine, albuterol, alprazolam, amitriptyline, amobarbital, amoxapine, antipyrine, atenolol, atropine, azatadine, baclofen, benzocaine, bromocriptine, brompheniramine, brotizolam, bupivacaine, buspirone, butabarbital, butalbital, caffeine, carbamazepine, cetirizine, chlorcyclizine, chlordiazepoxide, chlormezanone, chloroquine, chlorpheniramine, chlorpromazine, chlorpropamide, chlorprothixene, chlorthalidone, chlorzoxazone, cimetidine, cisapride, clomipramine, clonazepam, clonidine, clozapine, cocaine, codeine, colchicine, cyclizine, cyclobenzaprine, dantrolene, desipramine, diazepam, diclofenac, diflunisal, diltiazem, diphenhydramine, diphenidol, diphen-

oxylate, dipyridamole, disopyramide, dobutamine, doxapram, doxepin, droperidol, encainide, ethidium bromide, ethopropazine, fenoprofen, fentanyl, flavoxate, fluoxetine, fluphenazine, flurazepam, flurbiprofen, fluvoxamine, furosemide, glutethimide, glyburide, guaifenesin, haloperidol, homatropine, hydralazine, hydrochlorothiazide, hydrocodone, hydromorphone, hydroxychloroquine, hydroxyzine, ibuprofen, imipramine, indomethacin, ketoconazole, ketoprofen, ketorolac, labetalol, levorphanol, lidocaine, loratadine, lorazepam, lovastatin, loxapine, mazindol, mefenamic acid, meperidine, mephenytoin, mepivacaine, mesoridazine, metaproterenol, metformin, methadone, methdilazine, methocarbamol, methotrexate, methotrimeprazine, methoxamine, methyldopa, methylphenidate, metoclopramide, metolazone, metoprolol, metronidazole, midazolam, moclobemide, morphine, nadolol, nalbuphine, naloxone, naphazoline, naproxen, nifedipine, nizatidine, norepinephrine, nortriptyline, oxazepam, oxycodone, oxymetazoline, paroxetine, pemoline, pentazocine, pentobarbital, pentoxifylline, perphenazine, pheniramine, phenobarbital, phenol, phenolphthalein, phentolamine, phenylbutazone, phenyltoloxamine, phenytoin, pimozide, pindolol, piroxicam, pramoxine, prazepam, prazosin, probenecid, procainamide, procaine, prochlorperazine, procyclidine, promazine, promethazine, propafenone, propantheline, propiomazine, propofol, propranolol, protriptyline, quazepam, quinidine, quinine, racemethorphan, ranitidine, remoxipride, risperidone, salicylic acid, scopolamine, secobarbital, sertraline, sotalol, sulfinpyrazone, sulindac, temazepam, terbutaline, terfenadine, tetracaine, theophylline, thiethylperazine, thiopental, thioridazine, thiothixene, timolol, tocainide, tolbutamide, tolmetin, trazodone, triamterene, triazolam, trifluoperazine, triflupromazine, trimeprazine, trimethoprim, trimipramine, verapamil, warfarin, xylometazoline, yohimbine, zopiclone

KEY WORDS
details of plasma extraction

REFERENCE
Koves,E.M. Use of high-performance liquid chromatography-diode array detection in forensic toxicology, *J.Chromatogr.A*, **1995**, *692*, 103–119.

SAMPLE
Matrix: urine

Sample preparation: 2 mL Urine + 0.5 g solid buffer I (pH 5-5.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and vortex it with 2 mL 5% aqueous lead acetate for 10 s, centrifuge at 600 g for 5 min, remove and keep organic phase. 2 mL Urine + 0.5 g solid buffer II (pH 9-9.5), vortex 15 s, add 4 mL ethyl acetate, agitate for 10 min, centrifuge at 600 g for 5 min. Remove organic layer and combine it with previous organic layer. Evaporate to dryness at 50° under a stream of nitrogen, reconstitute in 300 μL 50 μg/mL β-hydroxyethyltheophylline in MeOH, inject 5 μL aliquot. (Solid buffer I was KH_2PO_4:Na_2HPO_4 99:1, solid buffer II was $NaHCO_3$:K_2CO_3 3:2.)

HPLC VARIABLES
Column: 250 × 4.6 HP LiChrosorb RP-18

Mobile phase: Gradient. MeCN:buffer from 15:85 at 2 min to 80:20 at 20 min (Buffer was 50 mM NaH_2PO_4 containing 16 mM propylamine hydrochloride, adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 5

Detector: UV 230

CHROMATOGRAM
Retention time: 17.5

Internal standard: β-hydroxyethyltheophylline (4.4)

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES
Extracted: furosemide, metolazone, amiloride, acetazolamide, chlorothiazide, hydrochlorothiazide, quinethazone, triamterene, hydroflumethiazide, chlorthalidone, dichlorphenamide, trichloromethiazide, methyclothiazide, benzthiazide, cyclothiazide, polythiazide, bendroflumethiazide, probenecid, canrenone, flumethiazide, bumetanide, ethacrynic acid

Noninterfering: acetaminophen, aspirin, caffeine, diflunisal, fenoprofen, ibuprofen, indomethacin, methocarbamol, naproxen, phenylbutazone, sulindac, tetracycline, theobromine, theophylline, tolmetin, trimethoprim, verapamil

REFERENCE

Cooper,S.F.; Massé,R.; Dugal,R. Comprehensive screening procedure for diuretics in urine by high-performance liquid chromatography, *J.Chromatogr.*, **1989**, *489*, 65–88.

SAMPLE

Matrix: urine

Sample preparation: Make 5 mL urine alkaline (pH 9-10), add 2 g NaCl, extract twice with 6 mL ethyl acetate. Combine the organic layers and evaporate them to dryness under a stream of nitrogen, reconstitute the residue in 200 μL MeCN/water, inject a 10-20 μL aliquot.

HPLC VARIABLES

Column: 100 × 4 5 μm SGE 100 GL-4 C18P (Scientific Glass Engineering)

Mobile phase: MeCN:MeOH:water:trifluoroacetic acid 15:15:70:0.5

Flow rate: 0.8 or 1

Injection volume: 10-20

Detector: MS, ZAB2-SEQ (VG), PSP source coupled to LC, source 250°, probe 240-260°, scan m/z 200-550 or UV 270

CHROMATOGRAM

Retention time: 9.4

Limit of detection: 50 ng (by MS)

OTHER SUBSTANCES

Extracted: probenecid, bumetanide, ethacrynic acid

REFERENCE

Ventura,R.; Fraisse,D.; Becchi,M.; Paisse,O.; Segura,J. Approach to the analysis of diuretics and masking agents by high-performance liquid chromatography-mass spectrometry in doping control, *J.Chromatogr.*, **1991**, *562*, 723–736.

SAMPLE

Matrix: urine

Sample preparation: Buffer urine to 4.9 by mixing with an equal volume of pH 4.9 200 mM sodium phosphate buffer. Inject a 40 μL aliquot onto column A with mobile phase A, after 3 min backflush the contents of column A onto column B with mobile phase B and start the gradient. At the end of the run re-equilibrate for 10 min.

HPLC VARIABLES

Column: A 20 × 4 5 μm Hypersil octadecylsilica ODS; B 200 × 4.6 5 μm Shiseido SG-120 polymer-based C18

Mobile phase: A water; B Gradient. MeCN:buffer from 7:93 to 15:85 over 3.5 min, to 50:50 over 8.5 min, maintain at 50:50 for 11 min (Buffer was 6.9 g $NaH_2PO_4.H_2O$ in 1 L water, pH adjusted to 3.1 with phosphoric acid.)

Flow rate: 1

Injection volume: 40

Detector: UV 230

CHROMATOGRAM

Retention time: 21.8

Limit of detection: 1000 ng/mL

OTHER SUBSTANCES

Extracted: acetazolamide, amiloride, bendroflumethiazide, benzthiazide, bumetanide, caffeine, carbamazepine, chlorothiazide, chlorthalidone, clopamide, dichlorfenamide, ethacrynic acid, furosemide, hydrochlorothiazide, metyrapone, probenecid, triamterene, trichlormethiazide

KEY WORDS

column-switching; optimum detection wavelengths vary for each drug

REFERENCE

Saarinen,M.; Sirén,H.; Riekkola,M.-L. A column switching technique for the screening of diuretics in urine by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1993**, *16*, 4063–4078.

SAMPLE

Matrix: urine

Sample preparation: 5 mL Urine + 50 μL 100 μg/mL 7-propyltheophylline in MeOH + 200 μL ammonium chloride buffer + 2 g NaCl, extract with 6 mL ethyl acetate by rocking at 40 movements/min for 20 min and centrifuging at 800 g for 5 min, repeat extraction, combine organic layers, evaporate to dryness at 40° under a stream of nitrogen. Reconstitute in 200 μL MeCN: water 15:85 and inject 20 μL aliquots. (Ammonium chloride buffer was 28 g ammonium chloride in 100 mL water with the pH adjusted to 9.5 with concentrated ammonia solution.)

HPLC VARIABLES

Column: 75 × 4.6 3 μm Ultrasphere ODS

Mobile phase: Gradient. MeCN:100 mM ammonium acetate adjusted to pH 3 with concentrated phosphoric acid. From 10:90 to 15:85 over 2 min to 55:45 over 3 min to 60:40 over 3 min. Kept at 60:40 for 1 min, decreased to 10:90 over 1 min and equilibrated at 10:90 for 2 min.

Flow rate: 1

Injection volume: 20

Detector: UV 240

CHROMATOGRAM

Retention time: 7.8

Internal standard: 7-propyltheophylline (4.5)

Limit of detection: 100 ng/mL

OTHER SUBSTANCES

Extracted: xipamide, bumetanide, acetazolamide, amiloride, bendroflumethiazide, buthiazide, benzthiazide, canrenone, caffeine, clopamide, chlorthalidone, cyclothiazide, diclofenamide, ethacrynic acid, furosemide, hydrochlorothiazide, morazone, piretanide, polythiazide, probenecid, torsemide, triamterene

Interfering: mesocarb

REFERENCE

Ventura,R.; Nadal,T.; Alcalde,P.; Pascual,J.A.; Segura,J. Fast screening method for diuretics, probenecid and other compounds of doping interest, *J.Chromatogr.A*, **1993**, *655*, 233–242.

SAMPLE

Matrix: urine

Sample preparation: Direct injection into column A with mobile phase A for 1 min then back flush onto column B with mobile phase B.

HPLC VARIABLES

Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 250 × 4 Hypersil ODS-C18

Mobile phase: A Water; B Gradient. MeCN:buffer 15:85 for 1.5 min then to 80:20 over 8 min. Keep at 80:20 for 2.5 min then re-equilibrate with 15:85. (Buffer was 50 mM NaH_2PO_4 + 1.4 mL propylamine hydrochloride per liter adjusted to pH 3 with concentrated phosphoric acid.)

Flow rate: 1

Injection volume: 50

Detector: UV 230

CHROMATOGRAM

Retention time: 10.8

Limit of detection: 20 ng/mL.

OTHER SUBSTANCES

Simultaneous: bumetanide, ethacrynic acid, acetazolamide, amiloride, bendroflumethiazide, chlorthalidone, cyclothiazide, furosemide, hydrochlorothiazide, probenecid, triamterene

REFERENCE

Campíns-Falco,P.; Herráez-Hernández,R.; Sevillano-Cabeza,A. Column-switching techniques for screening of diuretics and probenecid in urine samples, *Anal.Chem.*, **1994**, *66*, 244–248.

SAMPLE

Matrix: urine

Sample preparation: Inject 50 μL urine on to column A and elute to waste with mobile phase A, after 1.5 min elute the contents of column A on to column B with mobile phase B and start the gradient.

HPLC VARIABLES
Column: A 20 × 2.1 30 μm Hypersil ODS-C18; B 125 × 4 5 μm HP-LiChrospher 100 RP 18
Mobile phase: A water; B Gradient. MeCN:water from 0:100 to 60:40 over 3 min, maintain at 60:40 for 3.5 min
Flow rate: 1
Injection volume: 50
Detector: UV 230

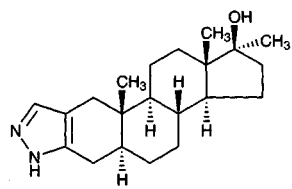
CHROMATOGRAM
Retention time: 7.2
Limit of detection: 20 ng/mL
Limit of quantitation: 70 ng/mL

OTHER SUBSTANCES
Extracted: metabolites, canrenone (UV 300)
Simultaneous: bendroflumethiazide, chlorthalidone, hydrochlorothiazide

KEY WORDS
column-switching

REFERENCE
Herráez-Hernández,R.; Soriano-Vega,E.; Campíns-Falcó,P. High-performance liquid chromatographic determination of spironolactone and its major metabolite canrenone in urine using ultraviolet detection and column-switching, *J.Chromatogr.B*, **1994**, *658*, 303–310.

Stanozolol



Molecular formula: $C_{21}H_{32}N_2O$
Molecular weight: 328.50
CAS Registry No.: 10418-03-8
Merck Index: 8951
Lednicer No.: 1 174

SAMPLE
Matrix: formulations
Sample preparation: Weigh out amount of powdered tablets equivalent to 3.36 mg stanozolol, add 20 mL EtOH:100 mM HCl 70:30, stir magnetically filter, repeat extraction. Combine the filtrates and make up to 50 mL with EtOH:100 mM HCl 70:30. Remove a 5 mL aliquot and add it to 1 mL 115 μg/mL 4-acetylbiphenyl in MeOH, make up to 10 mL with MeOH, inject a 10 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm Hypersil RP-18
Mobile phase: MeOH:50 mM $(NH_4)H_2PO_4$ 85:15
Flow rate: 1
Injection volume: 10
Detector: UV 230

CHROMATOGRAM
Retention time: 5.3
Internal standard: 4-acetylbiphenyl (4.2)

KEY WORDS
tablets

REFERENCE
Cavrini,V.; Di Pietra,A.M.; Augusta Raggi,M.; Grazia Maioli,M. Determination of stanozolol in tablets by derivative ultraviolet spectrophotometry and high-performance liquid chromatography, *Analyst*, **1987**, *112*, 1671–1674.

SAMPLE
Matrix: formulations
Sample preparation: Oils. 1 mL Sample + 25 mL MeOH:water 90:10, shake vigorously for 5 min, centrifuge, inject a 10 μL aliquot of the supernatant. Tablets. Grind a tablet to a fine powder, add 25 mL MeOH, sonicate for 5-10 min, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate. Suspensions (aqueous). Make up 5 mL to 50 mL with MeOH, filter (0.45 μm), discard first 5 mL of filtrate, inject a 10 μL aliquot of the remaining filtrate.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: MeOH:water 75:25
Flow rate: 1.5
Injection volume: 10
Detector: UV 240

CHROMATOGRAM
Retention time: 16.4
Limit of detection: 5 μg/mL

OTHER SUBSTANCES
Simultaneous: nandrolone propionate, methenolone acetate, testosterone propionate, aspirin, caffeine, formebolone, benzyl alcohol, testolactone, cortisone, fluoxymesterone, norethindrone, oxandrolone (UV 210), boldenone, ethisterone, methandrostenolone, nandrolone, norgestrel, testosterone, dehydroepiandrosterone (UV 210), mibolerone, methyltestosterone, methandriol (UV 210), norethindrone acetate, calusterone, mesterolone (UV 210), norethandrolone, benzyl benzoate, trenbolone acetate, nandrolone acetate
Interfering: testosterone acetate, oxymetholone

KEY WORDS
oils; tablets; suspensions

REFERENCE
Walters,M.J.; Ayers,R.J.; Brown,D.J. Analysis of illegally distributed anabolic steroid products by liquid chromatography with identity confirmation by mass spectrometry or infrared spectrophotometry, *J.Assoc.Off.Anal.Chem.*, **1990**, *73*, 904–926.

SAMPLE
Matrix: formulations
Sample preparation: Crush tablets, weigh out amount equivalent to 10 mg steroid, dissolve in 10 mL MeOH, sonicate for 15 min, filter. 1 mL Filtrate + 5 mL MeOH + 4 mL water, inject a 25 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Zorbax ODS
Mobile phase: Gradient. MeOH:water from 70:30 to 100:0 over 15 min, maintain at 100:0 for 15 min.
Flow rate: 1
Injection volume: 25
Detector: UV 240

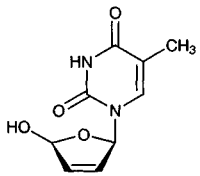
CHROMATOGRAM
Retention time: 15.5

OTHER SUBSTANCES
Simultaneous: boldenone, boldenone acetate, boldenone undecylenate, clostebol acetate, danazol (UV 280), fluoxymesterone, methandriol, methandriol-3-acetate, methandriol dipropionate, methandrostenolone, methyltestosterone, nandrolone, nandrolone decanoate, nandrolone phenylpropionate, nandrolone propionate, stanolone, testosterone, testosterone acetate, testosterone cypionate, testosterone enanthate, testosterone isobutyrate, testosterone propionate, testosterone undecanoate
Noninterfering: oxandrolone, oxymetholone, testosterone decanoate, testosterone isocaproate

KEY WORDS
tablets

REFERENCE
Lurie,I.S.rling,A.R.; Meyers,R.P. The determination of anabolic steroids by MECC, gradient HPLC, and capillary GC, *J.Forensic Sci.*, **1994**, *39*, 74–85.

Stavudine



Molecular formula: $C_{10}H_{12}N_2O_4$
Molecular weight: 224.22
CAS Registry No.: 3056-17-5
Merck Index: 8958

SAMPLE
Matrix: amniotic fluid, blood
Sample preparation: Condition a 3 mL Bond Elut C18 SPE cartridge with 2 mL MeOH and two 2 mL portions of water. 100 μL Plasma or amniotic fluid + 20 μL 10 μg/mL 3-hydroxyacetamidophenol in water, mix, add to the SPE cartridge, wash with two 2 mL portions of water, elute with 2 mL MeOH. Evaporate the eluate to dryness under reduced pressure, reconstitute with 100 μL MeCN:50 mM pH 3.3 ammonium phosphate buffer 6:94, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Ultrasphere ODS
Mobile phase: Gradient. A was MeCN:50 mM pH 3.3 ammonium phosphate buffer 6:94. B was MeCN:50 mM pH 3.3 ammonium phosphate buffer 25:75. A:B from 100:0 to 0:100 over 17 min, maintain at 0:100 for 5 min, return to initial conditions over 3 min, re-equilibrate for 17 min.
Flow rate: 1
Injection volume: 50
Detector: UV 266

CHROMATOGRAM
Limit of quantitation: 200 ng/mL

OTHER SUBSTANCES
Extracted: antipyrine

KEY WORDS
monkey; plasma; pharmacokinetics; SPE

REFERENCE
Odinecs,A.; Nosbisch,C.; Keller,R.D.; Baughman,W.L.; Unadkant,J.D. In vivo maternal-fetal pharmacokinetics of stavudine (2',3'-didehydro-3'-deoxythymidine) in pigtailed macaques (*Macaca nemestrina*), *Antimicrob.Agents Chemother.*, **1996**, *40*, 196–202.

SAMPLE
Matrix: blood

Sample preparation: Condition a 1 mL Bond Elut C18 SPE cartridge with 2 column volumes of MeOH and 2 column volumes of water. 250 μL Plasma + 100 μL 125 μg/mL IS in water, vortex, add to the SPE cartridge, wash with 2 column volumes of water, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 35°, reconstitute with 200 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Guard column: 37-53 μm pellicular ODS (Whatman)
Column: 250 × 4.6 5 μm Apex octadecyl (Jones Chromatography)
Mobile phase: MeOH:50 mM KH_2PO_4 20:80
Flow rate: 1
Injection volume: 50
Detector: UV 254

CHROMATOGRAM
Retention time: 6.1
Internal standard: thymidine oxetane (7.7)
Limit of detection: 50 ng/mL
Limit of quantitation: 100 ng/mL

KEY WORDS
rat; monkey; plasma; SPE; pharmacokinetics

REFERENCE
Kaul,S.; Dandekar,K.A.; Pittman,K.A. Analytical method for the quantification of 2',3'-didehydro-3'-deoxythymidine, a new anti-human immunodeficiency virus (HIV) agent, by high-performance liquid chromatography (HPLC) and ultraviolet (UV) detection in rat and monkey plasma, *Pharm.Res.*, **1989**, *6*, 895–899.

SAMPLE
Matrix: blood
Sample preparation: Condition a 3 mL Bakerbond C18 SPE cartridge with 2 mL MeOH and two 2 mL portions of water. Evaporate 50 μL 5 ng/mL didanosine in MeOH into the bottom of a tube at 60° under a stream of nitrogen, add 500 μL plasma, vortex for 10 s, add to the SPE cartridge, wash with 1 mL water, allow to dry under vacuum for 10 min, elute with two 500 μL portions of MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL mobile phase, vortex for 30 s, centrifuge at 800 g for 5 min, inject a 100 μL aliquot of the supernatant.

HPLC VARIABLES
Guard column: 4 × 4 LiChroCART 4-4 RP-8 (Merck)
Column: 300 × 3.9 10 μm μBondapak phenyl
Mobile phase: MeOH:5 mM pH 6.8 phosphate buffer 10:90
Flow rate: 1
Injection volume: 100
Detector: UV 265

CHROMATOGRAM
Retention time: 9
Internal standard: didanosine (10.5)
Limit of detection: 10 ng/mL

KEY WORDS
plasma; rat; human; pharmacokinetics; SPE

REFERENCE
Burger,D.M.; Rosing,H.; van Gijn,R.; Meenhorst,P.L.; van Tellingen,O.; Beijnen,J.H. Determination of stavudine, a new antiretroviral agent, in human plasma by reversed-phase high-performance liquid chromatography with ultraviolet detection, *J.Chromatogr.*, **1992**, *584*, 239–247.

SAMPLE
Matrix: blood

Sample preparation: 200 μL Plasma + 1 mL ice-cold MeCN, mix vigorously for 30 s, centrifuge at 9000 g for 7 min. Remove the supernatant and add it to excess crystalline magnesium sulfate, mix for 2 min, centrifuge at 9000 g for 10 min. Remove the supernatant and evaporate it to dryness under a stream of nitrogen at room temperature, reconstitute the residue in 200 μL mobile phase, inject a 15-150 μL aliquot.

HPLC VARIABLES
Guard column: 25 × 2.3 PRP-1 (Hamilton)
Column: 250 × 4.1 10 μm PRP-1 (Hamilton)
Mobile phase: MeCN:5 mM pH 11.1 tetrabutylammonium hydroxide 16:84
Flow rate: 1.5
Injection volume: 15-150
Detector: UV 254

CHROMATOGRAM
Retention time: 5.5
Internal standard: stavudine

OTHER SUBSTANCES
Extracted: 4-deoxy-5-fluorouracil (UV 313), 5-fluorouracil, tegafur

KEY WORDS
plasma; rat; stavudine is IS

REFERENCE
Jarugula,V.R.; Boudinot,F.D. High-performance liquid chromatographic determination of 5-fluorouracil and its prodrugs, tegafur and 4-deoxy-5-fluorouracil, in rat plasma, *J.Chromatogr.B*, **1996**, *677*, 199–203.

SAMPLE
Matrix: blood, CSF, tissue
Sample preparation: Plasma. Mix 100 μL plasma with 50 μL 10% acetic acid and 500 μL 1 μg/mL IS in ethyl acetate, centrifuge at 9000 g for 3 min, remove a 400 μL aliquot of the organic layer, repeat the extraction with 400 μL fresh ethyl acetate. Combine the ethyl acetate extracts and evaporate them to dryness under a stream of nitrogen at 50° in less than 5 min, reconstitute with 100 μL mobile phase, inject an aliquot (J.Pharm.Sci. 1993, 82, 1232). Tissue, CSF. Homogenize nasal tissue with pH 7.4 phosphate buffer to form a 4% (w/v) mixture. Mix 300 μL CSF or tissue homogenate with 4 μL isotonic phosphate buffer at 37°. Remove 50 μL aliquot of the reaction mixture, add 50 μL MeOH, mix, centrifuge, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4 Lichrospher 100 RP-18e
Mobile phase: MeOH:water:acetic acid 16:84:0.2
Column temperature: 40
Flow rate: 1.0
Detector: UV 265

CHROMATOGRAM
Retention time: 4.2
Internal standard: 1-(2-deoxy-3,5-epoxy-β-D-threo-pentofuranosyl)thymine (5.4)

KEY WORDS
plasma; rat; pharmacokinetics; nose

REFERENCE
Yajima,T.; Juni,K.; Saneyoshi,M.; Hasegawa,T.; Kawaguchi,T. Direct transport of 2',3'-didehydro-3'-deoxythymidine (D4T) and its ester derivatives to the cerebrospinal fluid via the nasal mucous membrane in rats, *Biol.Pharm.Bull.*, **1998**, *21*, 272–277.

SAMPLE
Matrix: blood, urine

Sample preparation: Dilute 100 μL urine to 1 mL with water. Evaporate 20 μL 5 μg/mL IS in MeOH in the bottom of a tube at 45° under reduced pressure, add 1 mL plasma or diluted urine, add 8 mL dichloromethane:isopropanol 95:5, shake horizontally at 180 cycles/min for 10 min, centrifuge at 750 g. Remove the organic layer and evaporate it to dryness under reduced pressure at 45°, reconstitute the residue in 100 μL mobile phase, vortex, inject a 20 μL aliquot.

HPLC VARIABLES

Guard column: 20 mm long Supelguard LC-18 (Supelco)
Column: 150 × 4.6 5 μm Supelcosil LC-18
Mobile phase: MeCN:10 mM pH 4.60 $(NH_4)H_2PO_4$ 6.5:93.5 (7:93 for rabbit plasma)
Flow rate: 0.75
Injection volume: 20
Detector: UV 264

CHROMATOGRAM

Retention time: 9.7
Internal standard: thymidine oxetane (BMY-33644) (14.3)
Limit of detection: 12.2 (urine), 2.58 (plasma) ng/mL
Limit of quantitation: 35.2 (urine), 6.7 (plasma) ng/mL

KEY WORDS

human; rabbit; plasma; pharmacokinetics

REFERENCE

Wong,S.L.; Sawchuk,R.J. High-performance liquid chromatographic determination of 2',3'-didehydro-3'-deoxythymidine (d4T) in human and rabbit plasma and urine and its application to pharmacokinetic studies in the rabbit, *Pharm.Res.*, **1991**, *8*, 619–623.

SAMPLE

Matrix: blood, urine
Sample preparation: Plasma. Condition a 1 mL Bond Elut C18 SPE cartridge with MeOH and water. 500 μL Plasma + 50 μL IS solution, add to the SPE cartridge, wash with 2 column volumes of water, elute with 1 mL MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 37°, reconstitute the residue in 125 μL mobile phase, inject a 100 μL aliquot. Urine. Condition a 3 mL Bond Elut phenyl SPE cartridge with 3 mL MeOH and two column volumes of 20 mM pH 8.0 potassium phosphate buffer. 500 μL Urine + 50 μL IS solution, add to the SPE cartridge, wash with 1 column volume of 20 mM pH 4.1 potassium phosphate buffer, wash with 1 column volume of 20 mM pH 8.0 potassium phosphate buffer, wash with 1 column volume of water, elute with two 500 μL portions of MeOH:water 70:30 containing 1.4 mM triethylamine, dilute the eluate with 500 μL 20 mM pH 7.2 potassium phosphate buffer, vortex briefly, inject a 75 μL aliquot.

HPLC VARIABLES

Column: 250 × 4.6 5 μm Apex octadecyl (Jones)
Mobile phase: MeCN:10 mM ammonium phosphate 10:90 containing 7.2 mM triethylamine, pH adjusted to 2.5 with 85% phosphoric acid
Flow rate: 0.8
Injection volume: 75-100
Detector: UV 266

CHROMATOGRAM

Retention time: 7 (plasma), 7.5 (urine)
Internal standard: thymidine oxetane (9 (plasma), 10.5 (urine))
Limit of detection: 150 ng/mL (urine), 6 ng/mL (plasma)
Limit of quantitation: 500 ng/mL (urine), 25 ng/mL (plasma)

OTHER SUBSTANCES

Simultaneous: dapsone, fluconazole, gangiclovir, ketoconazole, sulfamethoxazole
Interfering: ethambutol

KEY WORDS

plasma; SPE; pharmacokinetics

REFERENCE
Janiszewski,J.S.; Mulvana,D.E.; Kaul,S.; Dandekar,K.A.; Barbhaiya,R.H. High-performance liquid chromatographic determination of 2',3'-didehydro-3'-deoxythymidine, a new anti-human immunodeficiency virus agent, in human plasma and urine, *J.Chromatogr.*, **1992**, *577*, 151–156.

SAMPLE
Matrix: solutions

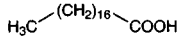
HPLC VARIABLES
Column: 220 × 4.6 5μm Brownlee C18
Mobile phase: MeOH:40 mM KH_2PO_4 containing 0.2% triethylamine 15:85, pH adjusted to 4.0 with 85% phosphoric acid (A) or MeCN:MeOH:40 mM KH_2PO_4 containing 0.2% triethylamine 3:15:85, pH adjusted to 4.0 with 85% phosphoric acid (B)
Column temperature: -10
Flow rate: 0.7
Injection volume: 25
Detector: UV 254

CHROMATOGRAM
Retention time: 26.3 (A), 16,5 (B)

OTHER SUBSTANCES
Simultaneous: didanosine

REFERENCE
Stancato,F.A.; Srinivas,N.R.; Knupp,C.A. Effect of temperature on the high-performance liquid chromatographic separation of the anti-HIV agents, didanosine and stavudine, *Biomed.Chromatogr.*, **1996**, *10*, 29–31.

Stearic acid



Molecular formula: $C_{18}H_{36}O_2$
Molecular weight: 284.48
CAS Registry No.: 57-11-4
Merck Index: 8959

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 2 mmoles stearic acid in 10 mL diethyl ether, add 10 mmoles 1-benzyl-3-p-tolyltriazene in 5 mL diethyl ether, stir at 36° for 3 h, cool, wash with two 5 mL portions of 10% HCl, wash with two 5 mL portions of 10% sodium carbonate, dry over anhydrous magnesium sulfate, evaporate to dryness, prepare a solution in mobile phase, inject an aliquot. (Synthesis of 1-benzyl-3-p-tolyltriazene is as follows. Stir 50.2 g p-toluidine in an ice/salt bath, add a mixture of 250 g crushed ice and 140 mL concentrated HCl, slowly add a solution of 46.8 g potassium nitrite in 150 mL water over 1-2 h until a positive starch/KI is obtained (stop addition 1-2 min before each test), stir for 1 h, allow to warm to 0°, adjust pH to 6.8-7.2 with cold concentrated sodium carbonate solution to give a solution of p-toluenediazonium chloride (Org.Syn., Coll.Vol. V, 797). Stir 107 g benzylamine in water in an ice/salt bath and slowly add the diazonium solution, extract with ether, recrystallize 1-benzyl-3-p-tolyltriazene from diethyl ether:n-hexane 50:50 to give yellow crystals (mp 77°) (Berichte 1888, 21, 1016). (Caution! 1-Benzyl-3-p-tolyltriazene explodes when heated to 90-100°!))

HPLC VARIABLES
Column: 1830 mm long Corasil II
Mobile phase: Heptane:chloroform 50:50
Detector: UV 254

OTHER SUBSTANCES
Also analyzed: heptadecanoic acid, palmitic acid

KEY WORDS
derivatization; normal phase

REFERENCE
Politzer,I.R.; Griffin,G.W.; Dowty,B.J.; Laseter,J.L. Enhancement of ultraviolet detectability of fatty acids for purposes of liquid chromatographic-mass spectrometric analyses, *Anal.Lett.*, **1973**, *6*, 539–546.

SAMPLE
Matrix: bulk
Sample preparation: Dissolve 3 μmole compound in 125 μL dichloromethane, add 125 μL 72 mM O-(p-nitrobenzyl)-N,N'-diisopropylisourea in dichloromethane, heat at 80° for 2 h, cool, inject an aliquot. (Synthesis of O-(p-nitrobenzyl)-N,N'-diisopropylisourea is as follows. Mix 15.3 g 4-nitrobenzyl alcohol, 12.6 g diisopropylcarbodiimide, 10 mg copper(II) chloride, and 10 mL DMF, let stand at room temperature for 96 h, remove the solvent by distillation (45°/10 mm Hg), dissolve the residue in petroleum ether (bp 40-80°), chromatograph on a 200 × 30 column of aluminum oxide, elute with petroleum ether until the eluate does not turn moistened litmus paper blue. Remove the petroleum ether under reduced pressure to give O-(p-nitrobenzyl)-N,N'-diisopropylisourea as yellow crystals (mp 42° (softening after 38°)) (Liebigs Ann. Chem. 1965, 685, 161).

HPLC VARIABLES
Column: 250 mm long 5 μm MicroPak silica
Mobile phase: Hexane:chloroform 80:20
Detector: UV 254

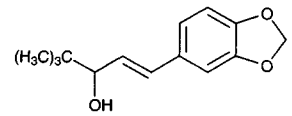
CHROMATOGRAM
Limit of detection: 4 pmole

OTHER SUBSTANCES
Also analyzed: lauric acid, myristic acid, palmitic acid

KEY WORDS
derivatization; normal phase

REFERENCE
Knapp,D.R.; Krueger,S. Use of O-p-nitrobenzyl-N,N'-diisopropylisourea as a chromogenic reagent for liquid chromatographic analysis of carboxylic acids, *Anal.Lett.*, **1975**, *8*, 603–610.

Stiripentol



Molecular formula: $C_{14}H_{18}O_3$
Molecular weight: 234.29
CAS Registry No.: 49763-96-4

SAMPLE
Matrix: blood
Sample preparation: 300 μL Blood + 15 μg piperonyl alcohol + 3 mL ethyl acetate, extract, centrifuge at 3000 rpm for 10 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue with three 50 μL portions of anhydrous toluene, add 2 μL triethylamine, add 0.5 μL di-n-butyltin dilaurate, add 5 μL phenyl isocyanate, let stand overnight at room temperature, add EtOH, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Bakerbond DNBPG chiral

Mobile phase: Hexane:isopropanol 95:5
Flow rate: 2
Detector: F ex 290 em 355

CHROMATOGRAM
Retention time: 6.4 (S), 7.3 (R)
Internal standard: piperonyl alcohol (9.0)
Limit of detection: 8 ng/mL

KEY WORDS
derivatization; rat; whole blood; pharmacokinetics; chiral

REFERENCE
Zhang,K.; Tang,C.; Rashed,M.; Cui,D.; Tombret,F.; Botte,H.; Lepage,F.; Levy,R.H.; Baillie,T.A. Metabolic chiral inversion of stiripentol in the rat. I. Mechanistic studies, *Drug Metab.Dispos.*, **1994**, *22*, 544–553.

Streptokinase

CAS Registry No.: 9002-01-1
Merck Index: 8981

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 75 × 7.5 Bio-Gel TSK-phenyl-5-PW
Mobile phase: Gradient from 100 mM pH 7.0 to water over 11 min (?)
Flow rate: 1
Detector: UV 280

CHROMATOGRAM
Retention time: 7

REFERENCE
Jackson,K.W.; Malke,H.; Gerlach,D.; Ferretti,J.J.; Tang,J. Active streptokinase from the cloned gene in Streptococcus sanguis is without the carboxyl-terminal 32 residues, *Biochem.*, **1986**, *25*, 108–114.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: Bakerbond C4
Mobile phase: Gradient. MeCN:0.1% trifluoroacetic acid from 0:100 to 60:40 over 90 min
Flow rate: 1
Injection volume: 200
Detector: UV 210

OTHER SUBSTANCES
Also analyzed: alteplase, urokinase, anistreplase

REFERENCE
Werner,R.G.; Bassarab,S.; Hoffmann,H.; Schlüter,M. Quality aspects of fibrinolytic agents based on biochemical characterization, *Arzneimittelforschung*, **1991**, *41*, 1196–1200.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 600 × 7.5 Spherogel 3000 SW
Mobile phase: 230 mM pH 6.8 phosphate buffer containing 0.1% sodium dodecyl sulfate
Flow rate: 0.5
Detector: UV 280

CHROMATOGRAM
Retention time: 27

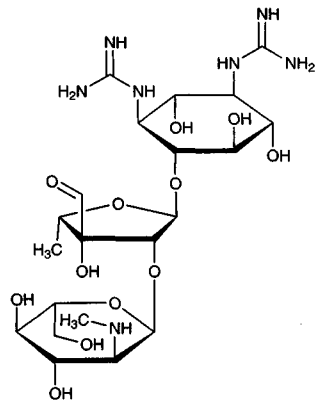
OTHER SUBSTANCES
Simultaneous: aggregates
Also analyzed: anistreplase, urokinase, alteplase

KEY WORDS
SEC; GPC

REFERENCE
Werner,R.G.; Bassarab,S.; Hoffmann,H.; Schlüter,M. Quality aspects of fibrinolytic agents based on biochemical characterization, *Arzneimittelforschung*, **1991**, *41*, 1196–1200.

Streptomycin

Molecular formula: $C_{21}H_{39}N_7O_{12}$
Molecular weight: 581.58
CAS Registry No.: 57-92-1, 3810-74-0 (sulfate)
Merck Index: 8983



SAMPLE
Matrix: blood
Sample preparation: Condition a Sep-Pak C18 SPE cartridge with 20 mL MeOH and 20 mL water. 400 µL Serum + 2 mL buffer + 100 µL 100 µg/mL dihydrostreptomycin sulfate in water, add to the SPE cartridge, wash with 2 mL water, centrifuge at 2300 g for 5 min, elute with 5 mL MeOH. Concentrate the eluate to 200 µL under vacuum at 30°, add 200 µL mobile phase, inject a 100 µL aliquot. (Buffer was 50 mM sodium 1-hexanesulfonate and 25 mM Na_3PO_4, pH adjusted to 2.0 with phosphoric acid.)

HPLC VARIABLES
Guard column: 30 × 4.6 10 µm Spheri-10 RP-8 (Brownlee)
Column: 250 × 4 5 µm LiChrosorb RP-18
Mobile phase: MeCN:buffer 8:92 (Buffer was 3.76 g sodium 1-hexanesulfonate and 9.50 g $Na_3PO_4.12H_2O$ in 900 mL water, adjust pH to 3.0 with phosphoric acid, make up to 1 L with water.)
Column temperature: 55
Flow rate: 1
Injection volume: 100
Detector: UV 195

CHROMATOGRAM
Retention time: 18
Internal standard: dihydrostreptomycin (20)

Limit of detection: 2000 ng/mL

KEY WORDS
serum; SPE

REFERENCE
Kurosawa,N.; Kuribayashi,S.; Owada,E.; Ito,K.; Nioka,M.; Arakawa,M.; Fukuda,R. Determination of streptomycin in serum by high-performance liquid chromatography, *J.Chromatogr.*, **1985**, *343*, 379–385.

SAMPLE
Matrix: blood
Sample preparation: 100 μL Serum + 50 μL 3.5% perchloric acid, vortex for a few s, centrifuge at 10000 g for 1 min, inject a 50 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 8 10 μm Radial-PAK C18 radial compression (Waters)
Mobile phase: MeCN:water 20:80 containing 20 mM disodium 1,2-ethanedisulfonate, 5 mM sodium octanesulfonate, and 5 mM ninhydrin, adjusted to pH 3.3 with acetic acid
Flow rate: 1.5
Injection volume: 50
Detector: F ex 302 em 420 (cut-off filter) following post-column reaction. The column effluent mixed with 300 mM NaOH pumped at 0.5 mL/min and flowed through a 10 m × 0.5 mm i.d. stainless steel coil at 80° to the detector.

CHROMATOGRAM
Retention time: 12
Limit of detection: 1 μg/mL

KEY WORDS
serum; post-column reaction

REFERENCE
Kubo,H.; Li,H.; Kobayashi,Y.; Kinoshita,T. Fluorometric determination of streptomycin in serum by high-performance liquid chromatography using mobile phase containing fluorogenic reagent, *Anal.Biochem.*, **1987**, *162*, 219–223.

SAMPLE
Matrix: bulk
Sample preparation: Prepare a 4 mg/mL a solution in water, dilute a 3 mL aliquot to 50 mL with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeCN:buffer 8:92, pH 6.0 (Prepare by dissolving 3.8 g sodium 1-hexanesulfonate and 9.5 g Na_3PO_4 in 850 mL water and 80 mL MeCN, adjust pH to 6.0 with phosphoric acid, make up to 1 L with water. (Connect a 250 × 4.6 column of Bondapak C18/Corasil or Co:Pell ODS between pump and injector. Flush column with MeOH:water 50:50 at the end of the day.)
Column temperature: 25
Flow rate: 1.3
Injection volume: 25
Detector: UV 195

CHROMATOGRAM
Retention time: 21

OTHER SUBSTANCES
Simultaneous: dihydrostreptomycin, impurities

REFERENCE
Whall,T.J. Determination of streptomycin sulfate and dihydrostreptomycin sulfate by high-performance liquid chromatography, *J.Chromatogr.*, **1981**, *219*, 89–100.

SAMPLE

Matrix: milk, tissue

Sample preparation: Tissue. Condition a 5 mL 500 mg 40 μm Bakerbond aromatic sulfonic acid SPE cartridge with 5 mL water. Homogenize (Polytron Model PT 10/35) 5 g tissue with 10 mL 3.6% perchloric acid for 10-15 s, shake horizontally for 5 min, centrifuge at 2000 g for 5 min, add the supernatant to the SPE cartridge at 2 mL/min, wash with 3 mL water, allow to dry, elute with 9 mL buffer. Add 500 μL 200 mM 1-hexanesulfonic acid in water and 150 μL perchloric acid to the eluate, make up to 10 mL with water, filter (0.45 μm), inject a 2 mL aliquot of the filtrate on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B. Milk. 10 g Milk + 3 mL 3.6% perchloric acid, shake horizontally for 30 s, centrifuge at 2000 g for 5 min. Add the supernatant to 70 μL 5 M NaOH and 500 μL 200 mM 1-hexanesulfonic acid, make up to 10 mL with water, filter (0.45 μm), inject a 2 mL aliquot of the filtrate on to column A and elute to waste with mobile phase A, after 5 min elute the contents of column A on to column B with mobile phase B, after 5 min remove column A from the circuit, elute column B with mobile phase B, monitor the effluent from column B (J. AOAC Int. 1994, 77, 765). (Prepare the buffer by dissolving 33.46 g K_2HPO_4 and 1.05 g KH_2PO_4 in 1 L water, pH 8.0. At the end of each day wash with MeCN:water 50:50 at 1.5 mL/min for 10 min.)

HPLC VARIABLES

Column: A 40 × 4.6 5 μm Inertsil C8; B 250 × 4.6 5 μm LC8-DB (Supelco)

Mobile phase: A 10 mM 1-Hexanesulfonic acid adjusted to pH 3.3 with acetic acid; B MeCN: water 17:83 containing 10 mM 1-hexanesulfonic acid and 400 μM 1,2-naphthoquinone-4-sulfonic acid, adjust pH to 3.3 with acetic acid

Flow rate: A 1; B 1.5

Injection volume: 2000

Detector: F ex 347 em 418 (tissue) or ex 365 em 418 (milk) following post-column reaction. The column effluent mixed with 500 mM NaOH pumped at 0.5 mL/min and the mixture flowed through a 2 mL reaction coil (Pickering, Mountain View CA) at 50° to the detector. (At the end of each day wash the post-column reaction system with 1% acetic acid for 10 min and with water for 10 min.)

CHROMATOGRAM

Retention time: 22

Limit of detection: 10 ppb

OTHER SUBSTANCES

Extracted: dihydrostreptomycin

KEY WORDS

post-column reaction; column-switching; SPE; pig; cow; muscle; kidney

REFERENCE

Gerhardt,G.C.; Salisbury,C.D.C.; MacNeil,J.D. Determination of streptomycin and dihydrostreptomycin in animal tissue by on-line sample enrichment liquid chromatography, *J.AOAC Int.*, **1994**, *77*, 334–337.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Guard column: 10 μm RP-18

Column: 150 × 4.6 5 μm Supelcosil LC-8-DB

Mobile phase: MeOH:buffer 1.5:98.5 (Buffer was 10 mM sodium 1-pentanesulfonate, 56 mM sodium sulfate, and 7 mM acetic acid.)

Flow rate: 1.5

Detector: F ex 340 em 455 following post-column reaction with derivatization reagent pumped at 0.9 mL/min. (Derivatization reagent was commercially available (Pierce) or prepared by adding 2.5 mL 2-mercaptoethanol and 2.5 mL Brij-35 to 850 mg o-phthalaldehyde in 10 mL MeOH, mix until decolorization is complete, add 1 L buffer, filter (0.45 μm), and refrigerate until used. Buffer was prepared by adjusting pH of 250 mM boric acid to 9.5 with 5 M KOH.)

CHROMATOGRAM
Retention time: 6

OTHER SUBSTANCES
Simultaneous: dihydrostreptomycin, neomycin, paromomycin

KEY WORDS
post-column reaction

REFERENCE
Shaikh,B.; Allen,E.H.; Gridley,J.C. Determination of neomycin in animal tissues, using ion-pair liquid chromatography with fluorometric detection, *J.Assoc.Off.Anal.Chem.*, **1985**, *68*, 29–36.

SAMPLE
Matrix: tissue
Sample preparation: Condition a 6 mL 500 mg Bond Elut Certify II SPE cartridge with 3 mL MeCN, 1 mL water, and three 1 mL aliquots of buffer B, do not allow to dry. Homogenize (Ultra Turrax TP 18/10) 8 g Kidney or meat, 5 mL buffer A, and 1 mL 85% trichloroacetic acid in water for 6 s, centrifuge at 5000 rpm for 3 min, add 2 mL dichloromethane, mix for 6 s, centrifuge at 5000 rpm for 5 min. Remove a 7 mL aliquot of the supernatant and add it to 900 μL 4 M NaOH, blend, centrifuge at 4000 rpm for 5 min. Remove the upper layer and add it to 900 μL 500 mM phosphoric acid, adjust the pH to 5.5-5.8 with 1 M NaOH or 500 mM phosphoric acid, add 2.5 mL buffer B, add to the SPE cartridge at 1 mL/min, rinse out the tube with 1 mL buffer A, add the rinse to the SPE cartridge, wash with two 5 mL portions of buffer A, suck dry for 2 s after each wash, wash with three 5 mL portions of 25% ammonia, suck dry for 2 s after each wash, wash with two 1 mL portions of water, suck dry for 2 s after each wash, wash with 1 mL water, suck dry for 10 s, elute with two 1 mL portions of 20% formic acid in MeOH. Evaporate the eluate to dryness under a stream of nitrogen at 60°, reconstitute the residue in 200 μL MeOH, mix for 3-4 s, evaporate to dryness, reconstitute with 400 μL buffer A, add 200 μL chloroform, mix vigorously for 10 s, centrifuge for 3 min. Filter (Costar Spin X with 0.22 μm nylon membrane) the aqueous layer while centrifuging at 5600 g for 2 min, inject a 25 μL aliquot of the filtrate. (Prepare buffer A by dissolving 4.45 g sodium 1-heptanesulfonate and 1.8 g Na_2HPO_4 in 750 mL water, adjust pH to 5.9 with 5 M phosphoric acid, adjust pH to 5.5 with 500 mM phosphoric acid, make up to 1 L with water, adjust pH to 5.5 with 500 mM phosphoric acid. Prepare buffer B by dissolving 13.35 g sodium 1-heptanesulfonate and 1.8 g Na_2HPO_4 in 750 mL water, adjust pH to 5.9 with 5 M phosphoric acid, adjust pH to 5.5 with 500 mM phosphoric acid, make up to 1 L with water, adjust pH to 5.5 with 500 mM phosphoric acid.)

HPLC VARIABLES
Guard column: 20 × 4.6 5 μm Supelcosil LC-ABZ + Plus
Column: 150 × 4.6 5 μm Supelcosil LC-ABZ + Plus
Mobile phase: MeCN:buffer 30:70 (Prepare buffer by dissolving 8.65 g sodium octanesulfonate and 110 mg potassium 1,2-naphthoquinone-4-sulfonate in 750 mL water, make up to 1 L with water, filter (0.45 μm), adjust pH to 3.24 with 1 mL acetic acid.)
Column temperature: 31
Flow rate: 0.6 for 0.5 min, 0.9 for 4 min, 0.6 for 9 min
Injection volume: 25
Detector: F ex 375 em 412 following post-column reaction. The column effluent mixed with 300 mM NaOH pumped at 0.3 mL/min in a 1.2 μL vortex mixer (Kratos PCRS 520) and the mixture flowed through a 15 m × 0.5 mm ID knitted coil at 40° and a room temperature heat exchanger to the detector.

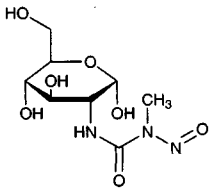
CHROMATOGRAM
Retention time: 17.3

OTHER SUBSTANCES
Extracted: dihydrostreptomycin

KEY WORDS
post-column reaction; muscle; kidney; SPE

REFERENCE
Hormazal,V.; Yndestad,M. High performance liquid chromatographic determination of dihydrostreptomycin sulfate in kidney and meat using post column derivatization, *J.Liq.Chromatogr.Rel.Technol.*, **1997**, *20*, 2259–2268.

Streptozocin



Molecular formula: $C_8H_{15}N_3O_7$
Molecular weight: 265.22
CAS Registry No.: 18883-66-4
Merck Index: 8991

SAMPLE
Matrix: blood, urine
Sample preparation: Plasma. Precipitate proteins with EtOH, centrifuge at 2000 rpm for 10 min. Remove the supernatant and adjust the pH to 4.0 with 6 M HCl, inject a 10 μL aliquot. Urine. Reconstitute lyophilized urine in MeOH:acetone 75:25, centrifuge, inject a 10 μL aliquot of the supernatant.

HPLC VARIABLES
Column: 100 × 2 Durapak 3,3'-oxypropionylnitrile
Mobile phase: Hexane:isopropanol 75:25
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 17
Limit of detection: 6 μg/mL

KEY WORDS
plasma; pharmacokinetics

REFERENCE
Adolphe,A.B.; Glasofer,E.D.; Troetel,W.M.; Weiss,A.J.; Manthei,R.W. Preliminary pharmacokinetics of streptozotocin, an antineoplastic antibiotic, *J.Clin.Pharmacol.*, **1977**, *17*, 379–388.

SAMPLE
Matrix: bulk, formulations
Sample preparation: Bulk. Prepare a 1 mg/mL solution in 100 mM pH 4.0 acetate buffer. Remove a 5 mL aliquot and add it to 5 mL 2 mg/mL potassium acid phthalate, mix, inject a 4 μL aliquot. Powders. Reconstitute the contents of a vial with 10-15 mL water, make up solution to 1 mL with water. Remove a 5 mL aliquot and add it to 5 mL 2 mg/mL potassium acid phthalate, mix, inject a 4 μL aliquot.

HPLC VARIABLES
Column: 300 × 4 10 μm μBondapak C18
Mobile phase: MeOH:water 3:97 containing 100 mM acetic acid, pH adjusted to 4.0 with 50% NaOH
Flow rate: 0.5
Injection volume: 4
Detector: UV 254

CHROMATOGRAM
Retention time: 5 (β-anomer), 7 (α-anomer)
Internal standard: potassium hydrogen phthalate (21)

OTHER SUBSTANCES
Noninterfering: degradation products

KEY WORDS
powders; mutarotation occurs in solution

REFERENCE
Oles,P.J. High-pressure liquid chromatographic separation and determination of anomeric forms of streptozocin in a powder formulation, *J.Pharm.Sci.*, **1978**, *67*, 1300–1302.

SAMPLE
Matrix: formulations
Sample preparation: Dilute with mobile phase, inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm cyano
Mobile phase: MeCN:20 mM sodium acetate 26:74, pH adjusted to 4.0 with acetic acid
Flow rate: 1.5
Injection volume: 20
Detector: UV 300

CHROMATOGRAM
Retention time: 2.01

OTHER SUBSTANCES
Simultaneous: granisetron (UV 300)

KEY WORDS
stability-indicating; injections; saline

REFERENCE
Mayron,D.; Gennaro,A.R. Stability and compatibility of granisetron hydrochloride in i.v. solutions and oral liquids and during simulated Y-site injection with selected drugs, *Am.J.Health-Syst.Pharm.*, **1996**, *53*, 294–304.

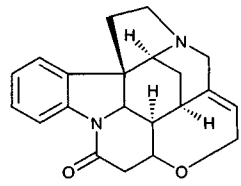
SAMPLE
Matrix: reaction mixtures
Sample preparation: If necessary, remove oxidizing power of solution by adding sodium metabisulfite, inject a 20 μL aliquot.

HPLC VARIABLES
Guard column: 15 × 4.6 5 μm Microsorb C8
Column: 250 × 4.6 5 μm Microsorb C8
Mobile phase: 200 mM pH 4.4 KH_2PO_4
Flow rate: 1
Injection volume: 20
Detector: UV 230

CHROMATOGRAM
Retention time: 9.8
Limit of detection: 500 ng/mL

REFERENCE
Lunn,G.; Rhodes,S.W.; Sansone,E.B.; Schmuff,N.R. Photolytic destruction and polymeric resin decontamination of aqueous solutions of pharmaceuticals, *J.Pharm.Sci.*, **1994**, *83*, 1289–1293.

Strychnine



Molecular formula: $C_{21}H_{22}N_2O_2$
Molecular weight: 334.42
CAS Registry No.: 57-24-9
Merck Index: 9020

SAMPLE

Matrix: blood, urine

Sample preparation: Add 1 mL whole blood or urine to Toxi-Tube A (Toxi-Lab, Irvine CA), add 3 mL water, mix by gentle inversion for 5 min, centrifuge at 1500 g for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 40°, reconstitute the residue with 50 µL MeCN:water 50:50, vortex for 10 s, centrifuge at 7500 g for 2 min, inject a 10 (urine) or 30 (blood) µL aliquot. (The detector wavelength shown is the wavelength of maximum absorbance. This will not necessarily be the optimal wavelength for the separation. Multiple wavelengths from 200-350 nm can be scanned using a diode-array detector. Otherwise, 220 nm may be a reasonable choice for initial work. Matrix may interfere.)

HPLC VARIABLES

Guard column: 20 mm long Symmetry C18
Column: 250 × 4.6 5 µm Symmetry C8 (Waters)
Mobile phase: Gradient. A was 50 mM pH 3.8 sodium phosphate buffer. B was MeCN. A:B 85:15 for 6.5 min, 65:35 for 18.5 min, 20:80 for 3 min (step gradient), re-equilibrate at initial conditions for 7 min.
Column temperature: 30
Flow rate: 1 for 6.5 min, to 1.5 over 18.5 min, maintain at 1.5 for 3 min (re-equilibrate at 1.5 mL/min)
Injection volume: 10-30
Detector: UV 207.5

CHROMATOGRAM

Retention time: 9.202

KEY WORDS

whole blood

REFERENCE

Gaillard,Y.; Pépin,G. Use of high-performance liquid chromatography with photodiode-array UV detection for the creation of a 600-compound library. Application to forensic toxicology, *J.Chromatogr.A*, **1997**, *763*, 149–163.

SAMPLE

Matrix: solutions

HPLC VARIABLES

Column: 250 × 4.6 Zorbax RX
Mobile phase: Gradient. A was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 1 L water. B was 10 mL concentrated orthophosphoric acid and 7 mL triethylamine in 200 mL water, make up to 1 L with MeCN. A:B from 100:0 to 0:100 over 30 min, maintain at 0:100 for 5 min.
Column temperature: 30
Flow rate: 2
Detector: UV 210

OTHER SUBSTANCES

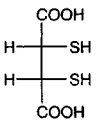
Also analyzed: acepromazine, acetaminophen, acetophenazine, albuterol, aminophylline, amitriptyline, amobarbital, amoxapine, amphetamine, amylocaine, antipyrine, aprobarbital, aspirin, atenolol, atropine, avermectin, barbital, benzocaine, benzoic acid, benzotropine, benzphetamine, berberine, bibucaine, bromazepan, brompheniramine, buprenorphine, buspirone,

butabarbital, butacaine, butethal, caffeine, carbamazepine, carbromal, chloramphenicol, chlordiazepoxide, chloroquine, chlorothiazide, chloroxylenol, chlorphenesin, chlorpheniramine, chlorpromazine, chlorpropamide, chlortetracycline, cimetidine, cinchonidine, cinchonine, clenbuterol, clonazepam, clonixin, clorazepate, cocaine, codeine, colchicine, cortisone, coumarin, cyclazocine, cyclobenzaprine, cyclothiazide, cyheptamide, cymarin, danazol, danthron, dapsone, debrisoquine, desipramine, dexamethasone, dextromethorphan, dextropropoxyphene, diamorphine, diazepam, diclofenac, diethylpropion, diethylstilbestrol, diflunisal, digitoxin, digoxin, diltiazem, diphenhydramine, diphenoxylate, diprenorphine, dipyrone, disulfiram, dopamine, doxapram, doxepin, dronabinol, ephedrine, epinephrine, epinine, estradiol, estriol, estrone, ethacrynic acid, ethosuximide, etonitazene, etorphine, eugenol, famotidine, fenbendazole, fencamfamine, fenoprofen, fenproporex, fentanyl, flubendazole, flufenamic acid, flunitrazepam, 5-fluorouracil, fluoxymesterone, fluphenazine, furosemide, gentisic acid, gitoxigenin, glipizide, glunixin, glutethimide, glybenclamide, guaiacol, halazepam, haloperidol, hydrochlorothiazide, hydrocodone, hydrocortisone, hydromorphone, hydroxyquinoline, ibogaine, ibuprofen, iminostilbene, imipramine, indomethacin, isocarbostyril, isocarboxazid, isoniazid, isoproterenol, isoxsuprine, ivermectin, ketamine, ketoprofen, kynurenic acid, levorphanol, lidocaine, lorazepam, lormetazepam, loxapine, mazindol, mebendazole, meclizine, meclofenamic acid, medazepam, mefenamic acid, megestrol, mepacrine, meperidine, mephentermine, mephenytoin, mephesin, mephobarbital, mepivacaine, mescaline, mesoridazine, methadone, methamphetamine, methapyrilene, methaqualone, methazolamide, methocarbamol, methoxamine, methsuximide, methyl salicylate, methyldopa, methyldopamine, methylphenidate, methylprednisolone, methyltestosterone, methyprylon, metoprolol, mibolerone, morphine, nadolol, nalorphine, naloxone, naltrexone, naphazoline, naproxen, nefopam, niacinamide, nicotine, niacin, nifedipine, niflumic acid, nitrazepam, norepinephrine, nortriptyline, noscapine, nylidrin, oxazepam, oxycodone, oxymorphone, oxyphenbutazone, oxytetracycline, papaverine, pargyline, pemoline, pentazocine, pentobarbital, persantine, phenacetin, phenazocine, phenazopyridine, phencyclidine, phendimetrazine, phenelzine, pheniramine, phenobarbital, phenothiazine, phensuximide, phentermine, phenylbutazone, phenylephrine, phenylpropanolamine, piperocaine, prazepam, prednisolone, primidone, probenecid, progesterone, propiomazine, propranolol, propylparaben, pseudoephedrine, puromycin, pyrilamine, pyrithyldione, quazepam, quinaldic acid, quinidine, quinine, ranitidine, recinnamine, reserpine, resorcinol, saccharin, albuterol, salicylamide, salicylic acid, scopolamine, scopoletin, sulfacetamide, sufadiazine, sulfadimethoxine, sulfaethidole, sulfamerazine, sulfamethazine, sulfamethoxizole, sulfanilamide, sulfapyridine, sulfasoxizole, sulindac, tamoxifen, temazepam, testosterone, tetracaine, tetracycline, tetramisole, thebaine, theobromine, theophylline, thiabendazole, thiamine, thiamylal, thiobarbituric acid, thioridazine, thiosalicylic acid, thiothixene, thymol, tolazamide, tolazoline, tobutamide, tolmetin, tranylcypromine, triamcinolone, tribenzylamine, trichloromethiazide, trifluoperazine, trihexyphenidyl, trimethoprim, tripelennamine, triprolidine, tropacocaine, tyramine, verapamil, vincamine, warfarin, yohimbine, zoxazolamine

REFERENCE

Hill,D.W.; Kind,A.J. Reversed-phase solvent gradient HPLC retention indexes of drugs, *J.Anal.Toxicol.*, **1994**, *18*, 233–242.

Succimer



Molecular formula: $C_4H_6O_4S_2$
Molecular weight: 182.22
CAS Registry No.: 304-55-2
Merck Index: 9034

SAMPLE

Matrix: solutions

Sample preparation: 100-200 μL Solution + 1.75-1.85 mL buffer + 50 μL reagent (final volume 2 mL), purge head space with nitrogen, shake at room temperature in the dark for 5 min, add 2 mL dichloromethane, shake for 10 s, centrifuge for 2 min. Remove the aqueous phase and adjust the pH to 7 with 15 μL 6 M HCl, inject a 20 μL aliquot. (Buffer was 100 mM pH 8.0 ammonium bicarbonate purged with nitrogen for at least 1 h before use. Reagent was 40 mM bromobimane in MeCN.)

HPLC VARIABLES
Guard column: 45 × 4.6 10 μm Ultrasphere IP C18
Column: 250 × 4.6 5 μm Ultrasphere IP C18
Mobile phase: Gradient. A was 20 mM tetrabutylammonium bromide in MeOH. B was 20 mM tetrabutylammonium bromide in water. A:B 55:45 for 11 min, to 75:25 in 1 min, maintain at 75:25 for 7 min, return to initial conditions over 1 min, re-equilibrate for 15 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 356 em 450

CHROMATOGRAM
Retention time: k' 2.55

OTHER SUBSTANCES
Simultaneous: 4-sec-butyl-5-ethyl-2-thiobarbituric acid, 2,3-dimercaptopropane-1-sulfonic acid, N-(2,3-dimercaptopropyl)phthalamidic acid, 2-mercaptoethanesulfonic acid, mercaptosuccinic acid, sodium sulfite

KEY WORDS
protect from light; derivatization

REFERENCE
Maiorino,R.M.; Weber,G.L.; Aposhian,H.V. Fluorometric determination of 2,3-dimercaptopropane-1-sulfonic acid and other dithiols by precolumn derivatization with bromobimane and column liquid chromatography, *J.Chromatogr.*, **1986**, *374*, 297–310.

SAMPLE
Matrix: urine
Sample preparation: 50 μL Urine + 900 μL buffer + 50 μL reagent, stir under nitrogen for 45 min, add 50 μL 80 mM monobromobimane in MeCN, let stand under nitrogen in the dark for 10 min, add 2 mL dichloromethane, extract for 15 s, centrifuge for 2 min, repeat extraction. Remove the aqueous phase and add 5 μL 6 M HCl, inject a 20 μL aliquot. (Buffer was 100 mM ammonium bicarbonate purged with nitrogen for 1 h. Reagent was 50 mM dithiothreitol in nitrogen-purged water.) [Procedures for electrolytic reduction and reduction with sodium borohydride are also described.]

HPLC VARIABLES
Column: 150 × 4.6 5 μm Spherisorb ODS RP-18
Mobile phase: Gradient. A was 20 mM tetrabutylammonium bromide in MeOH. B was 20 mM tetrabutylammonium bromide in 10 mM pH 4.1 acetate buffer. A:B 47.5:52.5 for 12 min, to 90:10 over 5 min, maintain at 90:10 for 7 min, re-equilibrate at initial conditions for 11 min.
Flow rate: 1
Injection volume: 20
Detector: F ex 356 em 450

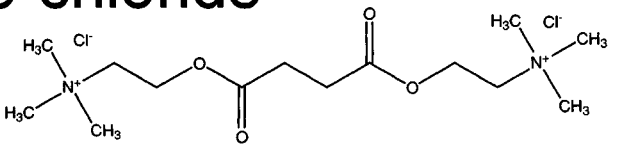
CHROMATOGRAM
Retention time: 10

KEY WORDS
pharmacokinetics; derivatization

REFERENCE
Maiorino,R.M.; Barry,T.J.; Aposhian,H.V. Determination and metabolism of dithiol-chelating agents: electrolytic and chemical reduction of oxidized dithiols in urine, *Anal.Biochem.*, **1987**, *160*, 217–226.

Succinylcholine chloride

Molecular formula: $C_{14}H_{30}Cl_2N_2O_4$
Molecular weight: 361.31
CAS Registry No.: 71-27-2, 6101-15-1 (dihydrate), 55-94-7 (bromnide), 541-19-5 (iodide)
Merck Index: 9044



SAMPLE
Matrix: formulations
Sample preparation: Inject a 10 μL aliquot.

HPLC VARIABLES
Column: 250 × 0.26 silica A (Perkin-Elmer)
Mobile phase: MeOH:water:500 mM tetramethylammonium sulfate 65:25:10
Flow rate: 1
Injection volume: 10
Detector: UV 214

CHROMATOGRAM
Retention time: 5.1
Limit of detection: 100 μg/mL

OTHER SUBSTANCES
Simultaneous: degradation products, choline, methyl p-hydroxybenzoate, succinic acid, succinylmonocholine

KEY WORDS
injections; saline

REFERENCE
Schmutz,C.W.; Mühlebach,S.F. Stability of succinylcholine chloride injection, *Am.J.Hosp.Pharm.*, **1991**, *48*, 501–506.

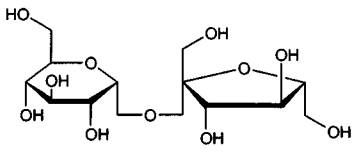
SAMPLE
Matrix: solutions
Sample preparation: Prepare a solution in saline, inject a 20 μL aliquot.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeOH:buffer 40-60 (Buffer was 20 mL Low-UV PIC B-7 (Waters) diluted with 480 mL water (10 mM 1-heptanesulfonic acid).)
Flow rate: 1
Injection volume: 20
Detector: UV 210

CHROMATOGRAM
Retention time: 7.8

REFERENCE
Woodman,T.F.; Johnson,B.; Marwaha,R.K. Determination of methacholine chloride by ion-pair high-pressure liquid chromatography, *J.Liq.Chromatogr.*, **1982**, *5*, 1341–1348.

Sucrose



Molecular formula: $C_{12}H_{22}O_{11}$
Molecular weight: 342.30
CAS Registry No.: 57-50-1
Merck Index: 9051

SAMPLE
Matrix: beverages, plants
Sample preparation: Beverages. Dilute 50-fold, filter (0.22 μm), inject an aliquot of the filtrate. Plants. Heat 1 g barley leaves and 10 mL EtOH:water 80:20 at 100° in a sealed tube for 15-30 min. Evaporate the liquid phase to dryness, reconstitute with water, pass through Analytichem trimethylaminopropyl and cyclohexyl SPE cartridges, inject an aliquot.

HPLC VARIABLES
Column: 300 × 6.5 Sugar-Pak I (Waters)
Mobile phase: water
Column temperature: 70
Flow rate: 0.4
Injection volume: 10
Detector: F ex 360 em 470 following post-column reaction. The effluent from the column passed through a 75 × 3.8 reactor containing Dowex 50 W × 2 sulfonic-acid type styrene divinylbenzene copolymer at 100° and mixed with 30 mM benzamidine in 1 M KOH pumped at 1 mL/min. This mixture flowed through a 530 μL reaction coil (Varian PCR-1) at 100° to the detector.

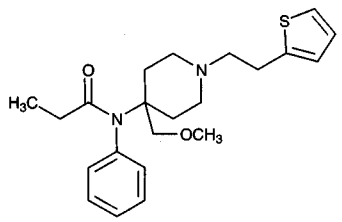
CHROMATOGRAM
Retention time: 15.30
Limit of detection: 22 pmole

OTHER SUBSTANCES
Extracted: dextrose, fructose

KEY WORDS
barley; SPE

REFERENCE
Coquet,A.; Haerdi,W.; Degli Agosti,R.; Veuthey,J.-L. Determination of sugars by liquid chromatography with post-column catalytic derivatization and fluorescence detection, *Chromatographia*, **1994**, *38*, 12–16.

Sufentanil



Molecular formula: $C_{22}H_{30}N_2O_2S$
Molecular weight: 386.56
CAS Registry No.: 56030-54-7, 60561-17-3 (citrate)
Merck Index: 9056
Lednicer No.: 3 118

SAMPLE
Matrix: blood, urine
Sample preparation: 50 μL Plasma or urine + 50 μL 4 M NaOH + 100 μL MeCN + 500 μL n-hexane, vortex for 30 s, centrifuge at 2000 rpm for 5 min. Remove the organic layer and evaporate it to dryness under a stream of nitrogen at 30°, reconstitute the residue in 100 μL mobile phase, inject a 50 μL aliquot.

HPLC VARIABLES
Column: 100 × 8 4 μm Nova pak cyano
Mobile phase: MeCN:5 mM pH 3.2 phosphate buffer 70:30
Flow rate: 2.5
Injection volume: 50
Detector: UV 214

CHROMATOGRAM
Retention time: 7.08
Limit of detection: 5 ng/mL

OTHER SUBSTANCES
Extracted: fentanyl, alfentanil

KEY WORDS
plasma

REFERENCE
Bansal,R.; Aranda,J.V. Simultaneous microassay of alfentanil, fentanil, and sufentanil by high performance liquid chromatography, *J.Liq.Chromatogr.*, **1995**, *18*, 339–348.

SAMPLE
Matrix: formulations
Sample preparation: Direct injection.

HPLC VARIABLES
Column: 100 × 3 Chromspher C18 (Chrompack)
Mobile phase: MeCN:MeOH:0.5% ammonium acetate in water 36.4:36.4:27.2
Flow rate: 0.6
Injection volume: 50
Detector: UV 225

CHROMATOGRAM
Retention time: 3

KEY WORDS
injections; saline; stability-indicating

REFERENCE
Roos,P.J.; Glerum,J.H.; Meilink,J.W. Stability of sufentanil citrate in a portable pump reservoir, a glass container and a polyethylene container, *Pharm.Weekbl.[Sci].*, **1992**, *14*, 196–200.

SAMPLE
Matrix: formulations
Sample preparation: 100 μL Injection solution + 400 μL 2.5 μg/mL haloperidol in water, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 100 × 4.6 5 μm Brownlee C18
Mobile phase: MeCN:MeOH:10 mM NaH_2PO_4 24:31:45, pH adjusted to 5.0 with 2 M KOH
Flow rate: 1.7
Injection volume: 100
Detector: UV 210

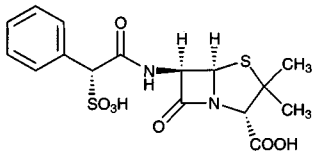
CHROMATOGRAM
Retention time: 15.46
Internal standard: haloperidol (9.74)

OTHER SUBSTANCES
Extracted: fentanyl

KEY WORDS
injections

REFERENCE
Dewell,W.M.,Jr.; Khandaghabadi,M.; D'Souza,M.J.; Solomon,H.M. High-performance liquid chromatographic determination of fentanyl and sufentanil returned from the operating room, *Am.J.Hosp.Pharm.*, **1993**, *50*, 2374–2375.

Sulbenicillin



Molecular formula: $C_{16}H_{18}N_2O_7S_2$
Molecular weight: 414.46
CAS Registry No.: 41744-40-5
Merck Index: 9059

SAMPLE
Matrix: blood
Sample preparation: Condition a Bond Elute-Certify II SPE cartridge with 5 mL MeOH:10% LiCl 40:60, 2 mL MeOH, and 2 mL water. Mix 500 μL plasma with 2 mL 50 mM ammonium acetate, add 100 μL 100 μg/mL carbenicillin in water. Add the mixture to the SPE cartridge, draw it through the cartridge under vacuum, wash with 3 mL MeCN:500 mM acetic acid 50:50 and 2 mL MeOH:100 mM ammonium acetate 50:50, elute with 1mL MeOH:10% LiCl 40:60. Inject a 40 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil C18-AR (Nacalai Tesque Co., Kyoto)
Mobile phase: MeOH:50 mM ammonium acetate 10:70
Flow rate: 0.9
Injection volume: 40
Detector: UV 254

CHROMATOGRAM
Internal standard: carbenicillin
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Noninterfering: probenecid

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Itoh,T.; Watanabe,N.; Ishida,M.; Tsuda,Y.; Koyano,S.; Tsunoi,T.; Shimada,H.; Yamada,H. Stereoselective disposition of sulbenicillin in humans, *Antimicrob.Agents Chemother.*, **1998**, *42*, 325–331.

SAMPLE
Matrix: blood, urine
Sample preparation: Condition a Bond Elut-SAX SPE cartridge with 2 mL MeOH and 2 mL water. Mix 500 μL plasma or diluted urine with 5 mL 50 mM ammonium acetate and 100 μL 100 μg/mL carbenicillin in water. Add the mixture to the SPE cartridge, draw it through the cartridge under vacuum, wash with 3 mL MeCN:500 mM acetic acid 50:50 and 2 mL MeOH:100 mM ammonium acetate 50:50, elute with 500 μL MeOH:10% LiCl 40:60. Inject a 20 μL aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm Cosmosil C18-AR (Nacalai Tesque Co., Kyoto)
Mobile phase: MeOH:50 mM pH 7.0 phosphate buffer 10:80

Flow rate: 0.9
Injection volume: 20
Detector: UV 254

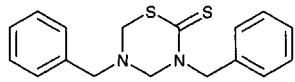
CHROMATOGRAM
Internal standard: carbenicillin
Limit of detection: 500 ng/mL

OTHER SUBSTANCES
Interfering: probenecid

KEY WORDS
plasma; pharmacokinetics; SPE

REFERENCE
Itoh,T.; Watanabe,N.; Ishida,M.; Tsuda,Y.; Koyano,S.; Tsunoi,T.; Shimada,H.; Yamada,H. Stereoselective disposition of sulbenicillin in humans, *Antimicrob.Agents Chemother.*, **1998**, *42*, 325–331.

Sulbentine



Molecular formula: $C_{17}H_{18}N_2S_2$
Molecular weight: 314.48
CAS Registry No.: 350-12-9
Merck Index: 9061

SAMPLE
Matrix: solutions
Sample preparation: Prepare a 1 mg/mL solution in MeOH, inject an aliquot.

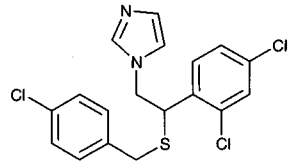
HPLC VARIABLES
Column: 125 × 4 5 μm LiChrospher 60 RP-Select-B (A) or 125 × 3 5 μm LiChrospher 60 RP-Select-B (B)
Mobile phase: MeCN: pH 2 trifluoroacetic acid 55:45
Flow rate: 1 (A) or 0.5 (B)
Detector: UV 290 (A), UV 240 (B)

CHROMATOGRAM
Retention time: 11.3 (A), 12.1 (B)

OTHER SUBSTANCES
Simultaneous: cloxyquin, chlorphenesin, naftifine, tolnaftate, degradation products

REFERENCE
Thoma,K.; Kübler,N.; Reimann,E. Untersuchung der Photostabilität von Antimykotica. 3. Mitteilung: Photostabilität lokal wirksamer Antimykotica [Photodegradation of antimycotic drugs. 3. Communication: Photodegradation of topical antimycotics], *Pharmazie*, **1997**, *52*, 362–373.

Sulconazole



Molecular formula: $C_{18}H_{15}Cl_3N_2S$
Molecular weight: 397.75
CAS Registry No.: 61318-90-9, 61318-91-0 (nitrate)
Merck Index: 9062
Lednicer No.: 3 133

SAMPLE

Matrix: blood
Sample preparation: 400 μL Plasma + 400 μL water + 50 μL 100 μg/mL miconazole in MeOH + 100 μL 1 M KOH + 6 mL hexane:dichloromethane 50:50, shake for 3 min, centrifuge at 4000 rpm for 6 min. Remove the upper organic layer and evaporate it to dryness under a stream of nitrogen, reconstitute the residue in 100 μL MeOH, inject a 25 μL aliquot.

HPLC VARIABLES

Guard column: 80 × 4 CoPell ODS
Column: 300 × 4 μBondapak C18
Mobile phase: MeCN:10 mM pH 8.0 NaH_2PO_4 buffer 66:34
Flow rate: 2
Injection volume: 25
Detector: UV 229

CHROMATOGRAM

Retention time: 7
Internal standard: miconazole (10)
Limit of quantitation: 500 ng/mL

KEY WORDS

plasma; dog; pharmacokinetics

REFERENCE

Fass,M.; Zaro,B.; Chaplin,M.; Matin,S. Reversed-phase high-pressure liquid chromatographic analysis of sulconazole in plasma, *J.Pharm.Sci.*, **1981**, *70*, 1338–1340.

SAMPLE

Matrix: formulations
Sample preparation: Dissolve 0.5 g cream containing 1% sulconazole nitrate in 5 mL THF, add fluocinonide, dilute to 100 mL with MeOH, inject a 25 μL aliquot onto column A with mobile phase A and allow components to elute from column A to column B for 7 min. After 7 min remove column A from circuit, monitor effluent from column B. Back-flush column A with mobile phase B for 3 min, equilibrate column A with mobile phase A for 5 min before next injection.

HPLC VARIABLES

Column: A 30 × 4.6 10 μm Partisil-10-ODS-3; B 70 × 2.1 Whatman Co:Pell ODS + 250 × 4.6 10 μm Partisil-10-ODS-3
Mobile phase: A MeCN:water:acetic acid 48:51:1 containing 10 mM $KClO_4$; B MeOH:THF 75:25
Flow rate: A 1.5; B 1
Injection volume: 25
Detector: UV 236

CHROMATOGRAM

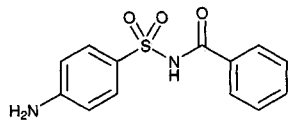
Retention time: 16
Internal standard: fluocinonide (12.5)

KEY WORDS

creams; column-switching

REFERENCE
Conley,D.L.; Benjamin,E.J. Automated high-performance liquid chromatographic column switching technique for the on-line clean-up and analysis of drugs in topical cream formulations, *J.Chromatogr.*, **1983**, *257*, 337–344.

Sulfabenzamide



Molecular formula: $C_{13}H_{12}N_2O_3S$
Molecular weight: 276.32
CAS Registry No.: 127-71-9
Merck Index: 9065
Lednicer No.: 2 112

SAMPLE
Matrix: milk, urine
Sample preparation: Urine. Filter (Rainin glassfiber microfilter and Rainin 0.45 μm nylon-66 filter), inject an aliquot. Milk. Filter (Rainin glassfiber microfilter), inject an aliquot.

HPLC VARIABLES
Column: 250 × 4.6 5 μm YMC-Pack ODS-AQ (YMC)
Mobile phase: MeOH:buffer 6:94 pH adjusted to 3.0 (Buffer was 70 mM in sodium dodecyl sulfate and 20 mM in NaH_2PO_4.)
Column temperature: 40
Detector: UV 254

CHROMATOGRAM
Retention time: 8.56

OTHER SUBSTANCES
Extracted: sulfacetamide, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxypyridazine, sulfamonomethoxine, sulfaquinoxaline, sulfathiazole, sulfisomidine

KEY WORDS
human; cow; micellar liquid chromatography

REFERENCE
Yang,S.; Khaledi,M.G. Micellar liquid chromatographic separation of sulfonamides in physiological samples using direct on-column injection, *J.Chromatogr.A*, **1995**, *692*, 311–318.

SAMPLE
Matrix: solutions
Sample preparation: Inject an aliquot of a solution in MeOH.

HPLC VARIABLES
Column: 300 × 3.9 μBondapak C18
Mobile phase: MeCN:water:acetic acid 12.5:86.5:1
Flow rate: 1.6
Injection volume: 10
Detector: UV 254

CHROMATOGRAM
Retention time: 26

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfachlorpyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfanilamide, sulfapyridine, sulfisoxazole

REFERENCE

Roos,R.W. High pressure liquid chromatographic determination of sulfisoxazole in pharmaceuticals and separation patterns of other sulfonamides, *J.Assoc.Off.Anal.Chem.*, **1981**, *64*, 851–854.

SAMPLE

Matrix: solutions
Sample preparation: Dissolve in MeOH:water 1:1 at a concentration of 50 μg/mL, inject a 10 μL aliquot.

HPLC VARIABLES

Column: 300 × 3.9 10 μm μBondapak C18
Mobile phase: MeOH:acetic acid:triethylamine:water 40:1.5:0.5:58
Flow rate: 1.5
Injection volume: 10
Detector: UV

CHROMATOGRAM

Retention time: k' 1.34

REFERENCE

Roos,R.W.; Lau-Cam,C.A. General reversed-phase high-performance liquid chromatographic method for the separation of drugs using triethylamine as a competing base, *J.Chromatogr.*, **1986**, *370*, 403–418.

SAMPLE

Matrix: solutions
Sample preparation: Prepare a solution in MeOH:water 25:75, inject a 5 μL aliquot.

HPLC VARIABLES

Column: 250 × 2.1 5 μm 201TP (Vydac)
Mobile phase: Gradient. A was MeCN containing 0.1% trifluoroacetic acid. B was water containing 0.1% trifluoroacetic acid. A:B from 5:95 to 40:60 over 20 min.
Flow rate: 0.2
Injection volume: 5
Detector: UV 270 or MS, Sciex API III triple quadrupole, IonSpray interface

CHROMATOGRAM

Retention time: 16.79

OTHER SUBSTANCES

Simultaneous: phthalylsulfathiazole, succinylsulfathiazole, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole

REFERENCE

Pleasance,S.; Blay,P.; Quilliam,M.A.; O'Hara,G. Determination of sulfonamides by liquid chromatography, ultraviolet diode array detection and ion-spray tandem mass spectrometry with application to cultured salmon flesh, *J.Chromatogr.*, **1991**, *558*, 155–173.

SAMPLE

Matrix: solutions
Sample preparation: Inject a 20 μL aliquot of a solution in MeOH:water 50:50.

HPLC VARIABLES

Guard column: 4 × 4 LiChrospher 5 μm 100 RP-18
Column: 250 × 4 5 μm Spherisorb ODS2
Mobile phase: MeCN:buffer 20:80 (Prepare buffer by dissolving 3.85 g ammonium acetate in 950 mL water, ad just pH to 4.00 with acetic acid, make up to 1 L with water.)
Column temperature: 35
Flow rate: 1

Injection volume: 20
Detector: UV 550 following post-column reaction. The column effluent mixed with ice-cold reagent pumped at 0.2 mL/min and the mixture flowed through a 25 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 20 mg/mL ammonium sulfamate in water pumped at 0.2 mL/min and this mixture flowed through an ice-cooled 200 cm × 0.33 mm ID coil. The effluent from this coil mixed with ice-cold 4 mg/mL N-(1-naphthyl)ethylenediamine hydrochloride in water pumped at 0.2 mL/min and this mixture flowed through a 60 cm × 0.33 mm ID coil to the detector. (Reagent was 800 mg sodium nitrite dissolved in 150 mL water and 50 mL concentrated HCl.)

CHROMATOGRAM
Retention time: 8.8
Limit of detection: 2 ppb

OTHER SUBSTANCES
Simultaneous: sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfamerazine, sulfamethazine, sulfamethoxazole, sulfamethoxypyridazine, sulfanilamide, sulfapyridine, sulfathiazole

KEY WORDS
post-column reaction; muscle; liver; kidney

REFERENCE
Guggisberg,D.; Mooser,A.E.; Koch,H. Screening method for the quantitative determination of twelve sulfonamides in meat, liver, and kidney by HPLC with online post-column derivatization, *Mitt.geb. Lebensmittelunters.Hyg.*, **1993**, *84*, 263–273.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeOH:buffer 0:100 at start of run, to 10:90 after injection (step gradient), to 12:88 over 30 min, to 18:82 over 5 min, to 30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

CHROMATOGRAM
Retention time: 52.5

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High-performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. I. Separations in methanol-modified solutions, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 365–381.

SAMPLE
Matrix: solutions

HPLC VARIABLES
Column: 300 × 0.35 5 μm Vydac IDI-TP C18 TMS capped
Mobile phase: Gradient. MeCN:MeOH:buffer 0:0:100 at start of run, to 0:5:95 after injection (step gradient), to 0:8:92 over 7 min, to 6:0:94 (step gradient), maintain at 6:0:94 for 14 min, to 0:16:84 over 5 min, to 0:18:82 over 5 min, to 0:30:70 over 5 min. (Buffer was 1 mM pH 2.72 phosphate buffer.)
Column temperature: 30
Flow rate: 0.006
Injection volume: 1
Detector: UV 270

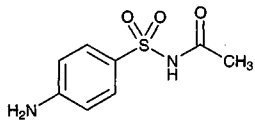
CHROMATOGRAM
Retention time: 47

OTHER SUBSTANCES
Simultaneous: diaveridine, phthalyl sulfathiazole, pyrimethamine, succinyl sulfathiazole, sulfacetamide, sulfachloropyridazine, sulfadiazine, sulfadimethoxine, sulfaguanidine, sulfamerazine, sulfameter, sulfamethazine, sulfamethizole, sulfamethoxazole, sulfamethoxypyridazine, sulfamoxole, sulfanilamide, sulfanilic acid, sulfapyridine, sulfaquinoxaline, sulfathiazole, sulfisomidine, sulfisoxazole, trimethoprim

KEY WORDS
capillary HPLC

REFERENCE
Ricci,M.C.; Cross,R.F. High performance liquid chromatographic analyses of sulphonamides and dihydrofolate reductase inhibitors. II. Separations in acetonitrile modified solutions, ternary gradient studies & flow programming, *J.Liq.Chromatogr.Rel.Technol.*, **1996**, *19*, 547–564.

Sulfacetamide



Molecular formula: $C_8H_{10}N_2O_3S$
Molecular weight: 214.25
CAS Registry No.: 144-80-9, 127-56-0 (Na salt), 6209-17-2 (Na salt monohydrate)
Merck Index: 9067
Lednicer No.: 1 123

SAMPLE
Matrix: blood
Sample preparation: 500 μL Plasma + 100 μL 6% trichloroacetic acid, vortex for 1 min, add 1 mL MeCN, centrifuge at 2500 g for 10 min. Remove the supernatant and add it to 5 mL dichloromethane, vortex for 1 min, shake for 10 min, centrifuge, inject a 100 μL aliquot of the aqueous phase. Analyze for free ceforanide by centrifuging serum at 3000 rpm for 20 min through an Amicon micropartition system with YMT membranes, 200 μL ultrafiltrate + 20 μL 1 mg/mL sulfacetamide, inject a 100 μL aliquot.

HPLC VARIABLES
Column: 250 mm long C18 (Alltech)
Mobile phase: MeOH:100 mM pH 4.0 sodium acetate buffer 10:90
Flow rate: 2
Injection volume: 100
Detector: UV 254

CHROMATOGRAM
Internal standard: sulfacetamide

OTHER SUBSTANCES
Extracted: ceforanide